ACTIVITIES OF RESPIRATORY ENZYMES DURING THE METAMORPHOSIS OF THE HOUSEFLY, MUSCA DOMESTICA LINNAEUS*

BY DANIEL LUDWIG AND MARY C. BARSA
DEPARTMENT OF BIOLOGY, FORDHAM UNIVERSITY

Agrell (1949) described total dehydrogenase activity and the specific activities of malic, citric, succinic and glutamic dehydrogenases as U-shaped during the metamorphosis of the blowfly, *Calliphora erythrocephala*. However, Ludwig and Barsa (1958) found that only malic and succinic dehydrogenases and the malic enzyme have U-shaped activity curves during this period in the mealworm, *Tenebrio molitor*.

In the present investigations, a study was made of the dehydrogenase respiratory enzymes of the housefly to compare their activities with those of other insects during metamorphosis.

MATERIAL AND METHODS

The insects used in this study were DDT-sensitive houseflies obtained from the Boyce Thompson Institute for Plant Research. The adults were kept at room temperature (approximately 25° C.) and fed diluted milk and sugar water. The eggs were laid on filter paper placed in the milk. They were removed daily and placed in the larval medium which consisted of animal pellets which had been powdered and soaked in tap water. The larvae were reared at room temperature and when they began to leave the food, they were placed on a piece of filter paper in a large petri dish. Insects, within 6 hours of puparium formation, were placed in labelled beakers and kept at 25° C. Immediately following puparium formation, they were designated as 0-day pupae, although they were probably in the prepupal stage.

The houseflies were washed in an alcohol solution, according to the procedure followed by Cotty (1956), to remove surface bacteria before homogenization. They were homogenized by means of a motor-driven glass homogenizer for 1 minute in the

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proper buffer. In all cases the buffers were adjusted to a pH of 7.4. The activities of succinic, malic, glucose, glutamic, alpha-glycerophosphate, lactic and isocitric dehydrogenases and the malic enzyme were determined by the Thunberg technique as given by Umbreit, Burris and Stauffer (1945, p. 126). Details of substrates, coenzymes, buffers and salts used in each enzymatic determination, as well as the procedure followed in preparing the Thunberg tubes are given by Ludwig and Barsa (1958). Samples of the same homogenate were used in the experimental tube and in the control. The rates of enzyme activity (1/time in minutes for 90 per cent decoloration of the methylene blue) were measured at 30° C. Activity values were obtained by subtracting the rate of the control from that of the experimental tube. Throughout all experiments, a minimum of 10 determinations were made.

OBSERVATIONS

Changes in the activities of the dehydrogenase enzymes during the metamorphosis of the housefly are shown in Table 1 and

TABLE 1.

Dehydrogenase Activity During the Metamorphosis of the Housefly. Activity is Expressed as 1/Time in Minutes for 90 Per Cent Decoloration of Methylene Blue. Readings Were Made at 30°C. (GPD is Alpha-glycerophosphate Dehydrogenase).

Stages	Dehydrogenase								
	Alcohol	Glucose	GPD I	GPD II	Isocitric	Lactic (total)	Malic	Malic enzyme	Succinic
Larva	0.041	0.004	0.047	.....	0.402	0.048	0.333	0.097	0.008
Puparium just formed	0.042	.....	0.013	.....	0.282	0.012	0.337	0.047	0.006
Pupa, 1-day	0.037	0.014	0.011	.....	0.255	0.018	0.312	0.061	0.004
Pupa, 2-day	0.024	0.013	0.004	.....	0.220	0.006	0.204	0.056	0.002
Pupa, 3-day	0.027	0.013	0.005	.....	0.197	0.005	0.179	0.063	0.004
Pupa, 4-day	0.025	0.013	0.003	0.004	0.194	0.002	0.262	0.060	0.005
Pupa, 5-day	0.024	0.013	0.015	0.009	0.158	0.004	0.461	0.058	0.013
Adult, just emerged	0.043	0.003	0.043	0.030	0.180	0.005	0.897	0.086	0.028

Figure 1. The activities of succinic, malic, total alpha-glycerophosphate and alcohol dehydrogenases and of the malic enzyme

followed U-shaped curves. Alpha-glycerophosphate II (enzyme not requiring DPN) was absent until the 4-day pupa and its activity greatly increased in the adult. The activity of lactic dehydrogenase was high (0.048) in the larva but it decreased rapidly and very little activity was observed in the latter part

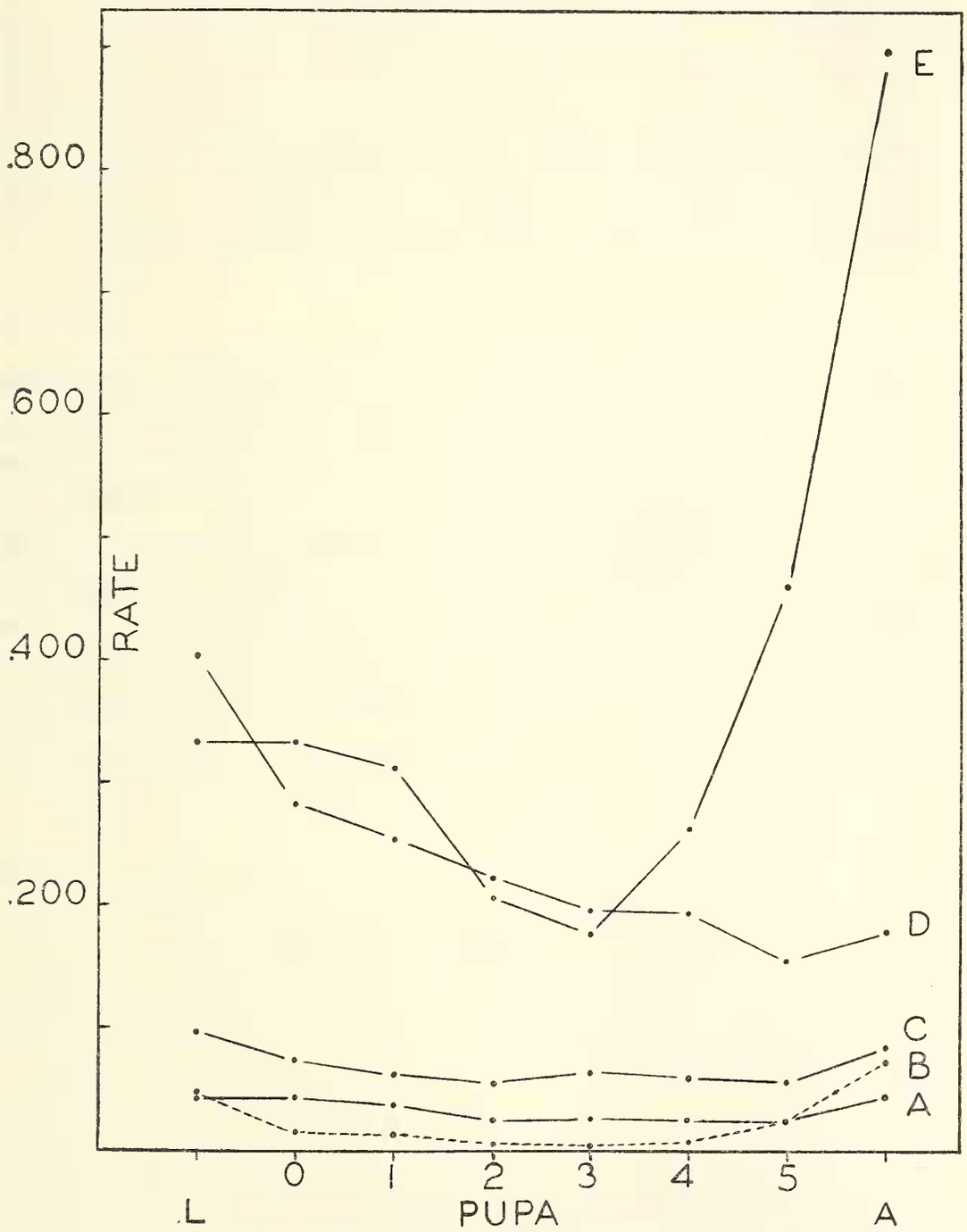

FIGURE 1. Changes in the activity of dehydrogenase enzymes during the metamorphosis of the housefly. Rate is expressed at 1/time in minutes for 90 per cent decoloration of methylene blue. Graph A, alcohol dehydrogenase; Graph B, alpha-glycerophosphate dehydrogenase; Graph C, malic enzyme; Graph D, isocitric dehydrogenase; Graph E, malic dehydrogenase. L, larva; A, newly emerged adult.

of the pupal stage or in the adult. A lactic dehydrogenase not requiring DPN was observed only in the larva. Its activity amounted to about one-fourth the total lactic dehydrogenase activity of this stage. A very low glucose dehydrogenase activity was observed during the prepupal and adult stages. However, this enzyme showed a constant value of 0.013 throughout the pupal stage. A low activity of glutamic dehydrogenase was obtained in the larva but it disappeared early in the pupal stage. Isocitric dehydrogenase activity was high at the beginning, but decreased steadily during the remainder of metamorphosis. The activities of malic and isocitric dehydrogenases were much greater than those of any of the other enzymes studied.

DISCUSSION

The activities of succinic, isocitric and malic dehydrogenases and the malic enzyme of the housefly are similar to those reported for these enzymes during the metamorphosis of the mealworm, *Tenebrio molitor,* by Ludwig and Barsa (1958). However, the malic dehydrogenase of the adult housefly is more active than that reported in the previous work for the adult mealworm. The activity curves for alcohol and alpha-glycerophosphate I (requiring DPN) dehydrogenases are U-shaped during the metamorphosis of the housefly but remained constant in the mealworm. Alpha-glycerophosphate II dehydrogenase (not requiring DPN) was not found until near the end of metamorphosis in the housefly but was present throughout this process in the mealworm. Glutamic dehydrogenase was found in the larva of the housefly but this enzyme does not appear until near the end of metamorphosis in the mealworm. In both species the activity of lactic dehydrogenase is very low throughout metamorphosis. These results differ from those of Agrell (1949) for the blowfly, *Calliphora erythrocephala,* in that the activity curves for glutamic and isocitric dehydrogenases of the housefly are not U-shaped. The activity curve for succinic dehydrogenase has been found to be U-shaped during the metamorphosis of the following species: *Drosophila melanogaster,* Wolsky (1941); *Calliphora erythrocephala,* Agrell (1949); *Bombyx mori,* Ito (1955); *Popillia japonica,* Ludwig and Barsa (1955); *Tenebrio molitor,* Ludwig and Barsa (1958); *Ephestia kühniella,* Diamantis (1959); and *Musca*

domestica, (the present work). Since the activity of this enzyme is very low, it could be a determining factor in the U-shaped respiratory curve characteristic of insect metamorphosis.

SUMMARY

A study was made of the activities of the dehydrogenase enzymes during the metamorphosis of the housefly using the Thunberg technique.

The activities of succinic, malic, total alpha-glycerophosphate and alcohol dehydrogenases and of the malic enzyme follow U-shaped curves during metamorphosis. Alpha-glycerophosphate II (not requiring DPN) was absent until the 4-day pupa and its activity greatly increased in the adult. Lactic and glutamic dehydrogenases were present in the larva but disappeared early in the pupal stage. Isocitric dehydrogenase activity was high at the beginning but decreased steadily during the remainder of metamorphosis. There was a low activity of glucose dehydrogenase in both the larval and adult stages. However, this enzyme showed a constant higher value throughout the pupal stage.

The activity curve for succinic dehydrogenase has been found to be U-shaped during metamorphosis in all insects studied. Since the activity of this enzyme is very low, it could be a determining factor in the U-shaped respiratory curve characteristic of insect metamorphosis.

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Natural History. The meeting was called to order by President Treat. Thirteen members and two guests were present.

The President reported on the publication schedule of the Journal, as discussed at the meeting of the Executive Committee. The first issue of 1957 will be out the middle of January, combined issues two and three by April, and the last issue by the middle of June.

Two new members—Master Tony Roberts, aged 14, and Master Bryan Treat, age 9—were unanimously elected to membership.

The President was about to bring the proposed changes in the By-laws to a vote when Dr. Forbes raised the point that they must first be advertised to the membership. It was agreed that a copy of the changes would be sent to each member previous to the meeting of November 19th. At that time they will be voted upon.

Dr. T. C. Schneirla of the American Museum of Natural History spoke on "Field Studies of Army Ants in Southeastern United States." This report on his summer's extension of the Arizona work with Doryline ants was illustrated by a series of kodachromes. Dr. Schneirla first reviewed his previous reports to the Society on the nomadic and statary phases of activity in these ants, and his work with the genus *Neiramyrmex* at the Museum's Southwestern Research Station in Arizona.

His summer's work was devoted to studying *Neiramyrmex nigrescens* in the Southeast, a common species extending to the Atlantic Coast. The study was made at the Bankhead National Forest in Alabama and the Sumter National Forest in South Carolina.

In Arizona Dr. Schneirla had always been able to physically follow the colony under observation. Not so in the Southeast. The "Tallulah" colony, at the end of the statary curve with a large oncoming pupal brood, was located in a stump and a cordon thrown around the bivouac. After five nights the colony was lost. It was again located and promptly lost. The colony had passed from statary to nomadic phase because of the stimulation of the maturing brood.

Next studied was the "Mound" colony and, like "Tallulah," was difficult to follow. However a method of tracery was used without keeping actual physical continuity with the colony. It was found that in the Southeast, colonies were better observed during the statary phase since at this time they selected stumps, while in Arizona they became subterranean. As with the Arizona observations of *nigrescens*, it was seen that the colony was unstable during the first few days of the 18-day statary phase, and that the queen became physogastric early and late in this phase.

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